In re: Milligan et al. Serial No.: 09/913,762

Filed: November 27, 2001

Page 3 of 7

In the Claims:

1. (Previously Presented) An assay for detecting an effect a compound has on a

membrane receptor, comprising the steps of:

a) adding the compound to a cell expressing a membrane receptor/reporter fusion

protein, the fusion protein comprising a membrane receptor segment and a reporter segment;

and

b) detecting any change of said receptor/reporter fusion protein by detecting a

signal from the reporter segment; wherein the membrane receptor segment is a constitutively

active mutant receptor.

2. (Original) The assay according to claim 1 wherein said assay is used to screen

compounds for their effect on membrane receptors.

3-4. (Canceled)

5. (Previously Presented) The assay according to claim 2 wherein any change is

detected as an increase in activity of the reporter segment of the fusion protein.

6. (Previously Presented) The assay according to claim 1 wherein said assay is used

to identify compounds that disrupt normal membrane receptor interactions.

7. (Previously Presented) The assay according to claim 1 for detecting a compound

which serves as an inverse agonist, antagonist or agonist of the membrane receptor.

8. (Previously Presented) The assay according to claim 7 wherein said inverse

agonist, antagonist or agonist of the membrane receptor is used in the study of receptor

function.

9. (Previously Presented) The assay according to claim 1 wherein said membrane

receptor is a G-protein coupled receptor.

In re: Milligan et al. Serial No.: 09/913,762

Filed: November 27, 2001

Page 4 of 7

a£.

10-11. (Canceled)

12. (Previously Presented) The assay according to claim 1 wherein the membrane

receptor/reporter fusion protein is initially unstable, such that the reporter activity is detected

at a basal level and wherein after binding of a compound to the receptor segment of the

fusion protein, the fusion protein is stabilized and an increase in reporter activity is observed.

13. (Previously Presented) The assay according to claim 9 wherein said G-protein

coupled receptor is a serotonin receptor.

14. (Previously Presented) The assay according to claim 1 wherein the

receptor/reporter fusion protein is expressed from nucleic acid construct comprising a gene

encoding said reporter segment which is fused in-frame to the 5' or 3' end of a gene encoding

said membrane receptor segment.

15. (Previously Presented) The assay according to claim 1 wherein the functionality

of said membrane receptor segment is substantially unaffected by fusion of the reporter

segment to the membrane receptor segment.

16. (Previously Presented) The assay according to claim 15 wherein said reporter

segment is Green Fluorescent Protein (GFP), or active variant thereof.

17. (Previously Presented) The assay according to claim 16 wherein light emitted by

said GFP protein is detected by fluorimetry, FACS, or microscopy techniques.

18. (Previously Presented) The assay according to claim 15 wherein said reporter

segment is Renilla reniformis (sea pansy) luciferase protein.

19. (Previously Presented) The assay according to claim 18 wherein said reporter

segment is luciferase which is detected in a microplate luminometer or using a CCD imaging

system.

In re: Milligan et al. Serial No.: 09/913,762

Filed: November 27, 2001

Page 5 of 7

رند

20. (Previously Presented) The assay according to claim 1 wherein the signal from

said reporter segment is used to localize and/or quantify the membrane receptor segment.

21. (Previously Presented) An assay according to claim 20 wherein any change of

said membrane receptor/reporter fusion protein is detected as a change in cellular localisation

of the receptor/reporter fusion protein, or semi-quantitatively by the synthesis or degradation

of said membrane receptor/reporter fusion protein.

22. (Previously Presented) An assay according to claim 1 wherein said detection of

any change of said membrane receptor/reporter fusion protein is carried out with cells placed

on the surface of a microscope slide.

23. (Previously Presented) The assay according to claim 1 wherein said detection of

any change of said membrane receptor/reporter fusion protein is carried out on cells placed in

a well of a microtitre plate.

24. (Previously Presented) An assay for detecting a test compound which has an

effect on a membrane receptor, comprising the steps of

a) expressing a membrane receptor/reporter fusion protein in a cell, wherein the

fusion protein comprises a membrane receptor segment and a reporter segment, and wherein

the membrane receptor segment is a constitutively active mutant receptor;

b) detecting a basal level of reporter activity;

c) adding a test compound to the cell; and

detecting a resulting activity of the reporter segment, wherein alteration of

reporter activity with respect to the basal level is due to the test compound having an effect

on the membrane receptor segment.

25–32. (Canceled)